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Minireview

Laser-Scanning Cytometry: A New Instrumentation with Many Applications â˜†

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Abstract

The laser-scanning cytometer (LSC) is a microscope-based cytofluorometer which has attributes of both flow and image cytometry. Laser-excited fluorescence emitted from fluorochromed individual cells on a microscope slide is measured at multiple wavelengths rapidly with high sensitivity and accuracy. Though the instrument has been available commercially for only 3 years, it is already used in a variety of different applications in many laboratories. This review focuses on the following unique analytical capabilities of LSC which complement those of flow cytometry and fluorescence image analysis: (a) the cells are positioned on slides during measurement so they may be examined repeatedly over time, a feature useful for studies of enzyme kinetics and other time-resolved processes; (b) sequential analysis of the same cells can be carried out using different immuno- or cytochemical stains or genetic probes, merging information on cell immunophenotype, cell functions, expression of particular proteins, DNA ploidy and cell cycle position, and/or cytogenetic profile for each measured cell; (c) any of the cells measured can be relocated to correlate with visual examination by fluorescence or brightfield microscopy or with any other parameter; (d) topographic distribution of fluorescence measurements within the cell, in cytoplasm vs nucleus, permits analysis of the translocation of regulatory molecules such as NFÎºB, p53, etc., and is essential for FISH analysis; (e) hyperchromicity of nuclear DNA as measured by maximal pixel fluorescence intensity allows one to identify cell types differing in degree of chromatin condensation such as mitotic or apoptotic cells; (f) analysis of tissue section architecture and of the constituents in transected cells within tissue sections by ratiometric assays normalized to DNA content extends applications of LSC in clinical pathology; (g) because cell loss during sample preparation and staining is minimal, samples with a paucity of cells can be analyzed; and (h) analyzed cells can be stored indefinitely, e.g., for archival preservation or additional analysis. Potential future applications of LSC are discussed.

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